Patent claims

1. Process for the cultivation and stimulation of three-dimensional, vital and mechanically-resistant cell transplants in a GMP-conform bioreactor with the process steps

a) the explant cells (12) taken from the organism and prepared for bioreactor cultivation using methods which are known and the carrier matrix (13) comprising commercially-available biocompatible, absorbable or autologous or homologous materials form a cell matrix suspension after being mixed

b) placed in a seeding piston (25) which is foiled if necessary and which has a cross-section which is adapted to the later transplant, it being hardened out or polymerized here,

c) if necessary, has minimum pressure applied to it by means of an exactly fitting, inert stamp (26)which is structured or foiled, if necessary,

d) the seeding piston with the transplant (11) is inserted in the chamber space of the bioreactor carcass,

e) where the transplant (11) from the seeding piston (25) is placed medially on the floor of the bioreactor and the bioreactor (1) is closed after the seeding piston has been removed,

f) where the transplant is subjected to further cultivation by feeding a perfusion flow into it and

g) the replacement tissue material is removed for further use after completion of the cultivation,

characterized in that the transplant is subjected to load in the cultivation phase on the surface opposite the floor of the bioreactor.

2. Process according to Claim 1, characterized in that the transplant is loaded by a stamp which applies pressure.

3. Process according to claims 1 und 2, characterized in that both the blending in the bioreactor chamber t due to the perfusion flow and the stamp which applies pressure to the transplant can be controlled with regard to time and quantity or density in relation to the cultivation conditions.

4. Process according to claims 1 - 3, characterized in that the transplant has conditioned cultivating medium flow through it at intervals and is subjected to loading in cycles by the stamp which applies pressure.

5. Process according to Claims 1 - 4, characterized in that the pressure load of the transplant takes place during the perfusion.

6. Process according to Claims 1 - 5, characterized in that the transplant is stimulated by using static, preferably in-vivo-similar pressure loads or construct deformations or they are continuously loaded by intermittent or dynamic pressure forces.

7. Process according to Claims 1 - 6, characterized in that the mechanical load is applied with a frequency exceeding 0.1 Hz.

8. Process according to claims 1 - 7 characterized in that the mechanical pressure stimulation is in the form of a symmetrical or asymmetrical half cosine or sine wave.

9. Process according to claims 1-8 characterized in that the pressure stamp which is from a magnetic material is moved longitudinal to the surface of the transplant in the bioreactor by an (electro-) magnetic field which is generated outside the bioreactor.

10. Process according to claims 1 - 9, characterized in that the magnetic field is generated by at least one permanent magnet.

11. Process according to claims 1-10 characterized in that at least two permanent magnets with alternating polarity are positioned on a mobile holder above the bioreactor and is driven by a servomotor in a cyclic manner, they thereby changing their position resulting in the pressure stamp applies pressure to the transplant alternately.

12. Process according to claims 1-8 characterized in that the coil of an electromagnet changes alters its current direction and voltage and therefore the field direction and magnetic flow density with high frequency via the bioreactor, resulting in the pressure stamp applying pressure to the transplant alternately.

13. Bioreactor for the cultivation and stimulation of three-dimensional, vital and mechanically resistant cell transplants in an GMP-conform bioreactor, characterized in that the bioreactor (1) of a basic carcass with a reactor lock (21) is connected in a pressure proof and sterile manner, this creating at least one reactor chamber, a storage space for a transplant (11) and a mini actuator (14) being implemented in this, the bioreactor (1) being equipped with at least two hose coupling connections (19) for the feeding and discharging of the medium in addition to the gassing.

14. Device according to Claim 13, characterized in that the cell culture constructs (11) can be directly or indirectly cultivated and stimulated on the bioreactor floor of a single-chamber bioreactor (1).

15. Device according to Claim 13 in that the cell culture constructs (11) are directly or indirectly, at least partially positioned on a floor of the upper reaction chamber of a double-chamber bioreactor for cultivation and stimulation, whilst this transplant (11) is in a second reactor chamber.

16. Device according to Claims 14 or 15, characterized in that the bioreactor (1) is equipped with a transplant insert on the floor of the upper reactor chamber in which the cell constructs (11) can be placed.

17. Device according to Claims 13 - 16, characterized in that the container of the bioreactor (1) is a cylinder-shaped corpus which is closed from above with a reactor lock (21).

18. Device according to Claims 13 - 17, characterized in that the reactor lock unit(s) (21) and the bioreactor (1) are connected by one threaded joint and at least one conical nipple (20) in such a way that the threaded joint is either created between the reactor lock (21) and the container(1) by a female thread in the container (1) and a male thread in the reactor lock working together or the threaded joint is created between the reactor lock (21) and the container (1) in that a male thread in the container (1) and a female thread in the reactor lock (21) work together.

19. Device according to Claims 13 - 18, characterized in that the reactor lock (21) in the form of a cover is

equipped with biosensors (9) and/or measuring heads (10).

20. Device according to Claims 13 - 19, characterized in that the cover (21) is equipped with a sample taking section (10).

21. Device according to Claims 13 - 20, characterized in that the basic carcass of the bioreactor (13) has at least two each of a feed and discharge borehole for the provision of hose coupling connections in the single-chamber bioreactor version.

22. Device according to Claims 13 - 20, characterized in that the basic carcass of the bioreactor (1) has at least two boreholes for the provision of hose coupling connections (19) in the double-chamber bioreactor version.

23. Device according to Claim 22, characterized in that at least one hose coupling connection (19) is integrated in the lower reaction chamber and at least one in the upper reaction chamber.

24. Device according to Claims 21 - 23, characterized in that the feed connections (19) and discharge connections (19) which enter the bioreactor chamber are fitted with a 3-way valve (6) or a 4-way valve (7) with a return function.

25. Device according to Claim 24, characterized in that at least one of the discharge connections (19) is provided with a sample taking section (10).

26. Device according to Claims 13 - 25, characterized in that the bioreactor (1) has a reactor floor of a completely or partially transparent material, preferably a transparent glass plate (17) for the monitoring of the transplant manufacture.

27. Device according to Claims 13 - 26, characterized in that a foil; fleece or membrane (18) of an antistatic or inert material is situated above the reactor floor of the bioreactor (1) for the positioning of the transplant (11), this material preferably being wide-meshed and light, fluid and gas permeable.

28. Device according to one of the Claims 13 - 27, characterized in that when in the version of a double-chamber reactor, the upper reactor chamber of the bioreactor (1) has an area which corresponds to the transplant area whilst the dimensions of the lower chamber are under those of the transplant (11) so that if a cell culture is placed medially, this construct is mainly positioned underneath the lower chamber and is partially on the reactor floor of the upper chamber.

29. Device according to Claim 28, characterized in that the space underneath the reactor chamber is filled out by a flat plate (16) of a biologically inert, light-permeating, wide-pored material, preferably of a porous sinter material so that this plate (16) is flush with the floor of the upper reactor chamber.

30. Device according to Claims 28 and 29, characterized in that a foil, fleece or membrane (18) of an antistatic or inert material is situated above the lower reactor chamber which is filled out by the plate (16) on the reactor floor of the upper reactor chamber of the double-chamber bioreactor (1), this material being for the positioning of the transplant (11, it preferably being wide-meshed and permeable to light, fluid and gas.

31. Device according to Claims 28-30, characterized in that the components which are underneath the transplant (11) in

the double-chamber bioreactor (1) such as the transparent plate (17), the lower chamber with the inserted porous material (16) and a wide-meshed membrane (18) have an overall height which does not exceed the focal distance of commercially available microscope and camera objectives.

32. Device according to Claim 13, characterized in that a magnetic, preferably piston-like mini actuator (14) is situated in the bioreactor (1) and can be moved through the bioreactor (1) in a controlled manner by one or more externally arranged control and steering magnets (15).

33. Device according to Claims 13-32, characterized in that the mini actuator (14) in the single-chamber bio reactor (1) is situated above the membrane (18) and the transplant (11) in the bioreactor space.

34. Device according to Claims 13-32, characterized in that the mini actuator (14) in the double-chamber bioreactor (1) is situated in the upper reactor space above the porous material (16), the membrane (18) and the transplant (11).

35. Device according to Claims 32-34, characterized in that the magnetic mini actuator (14) which is to be implemented comprises a magnetic nucleolus (22), preferably of a permanent magnet which is encapsulated in a biologically inert enveloping body (23), preferably plastic.

36. Device according to Claims 32-35, characterized in that the magnetic nucleolus (22) is so oriented that the field which is generated between the poles runs vertical to the transplant (11) so that the magnetic north pole of the complete mini actuator (14) is oriented in an upwards direction.

37. Device according to Claims 32-36, characterized in that

the biocompatible enveloping body (23) which surrounds the core magnets (22) has an external form which matches the form of the reactor chamber of the bioreactor (1).

38. Device according to Claims 32-35, characterized in that the complete height of the enveloping body (23) has been so selected that an implementation of the mini actuator (14) in the reactor space results in a vertically-oriented guiding of the pressure stamp of the mini actuator (14) towards the transplant (11).

39. Device according to Claims 32-38, characterized in that the piston-shaped mini actuator (14) comprises more than one enveloping body cylinder so that one of the enveloping body cylinders, preferably the upper one contains the encapsulated permanent magnet and an additional cylinder serves the stamp impression (24), whereby the spatially separated cylinders are joined by means of a bridge (34) or a connection which has the same function.

40. Device according to Claims 32-39, characterized in that the planar stamp surface (24) on the underside of the mini actuator (14) formed by the enveloping body (23, runs vertical to the guide direction in the bioreactor space (1).

41. Device according to Claims 32 and 40, characterized in that organotypical negative forms such as a convex form are embossed on the stamp surface (24) of the mini actuator (14).

42. Device according to Claims 32 and 41, characterized in that the planar or formed stamp surface (24) is embossed in the form of a grid structure in order to increase the stamp surface, this preferably having a small-meshed structure.

43. Device according to Claims 32 - 42, characterized in

that the enveloping body (24) of the mini actuator (14) is to be so provided with drill holes and/or flow channels (33) that a continued exact vertical guiding of the mini actuator (14) is still guaranteed at 3 points.

44. Device according to Claims 32 - 44, characterized in that the stamp surface (24) of the mini actuator (14) is fitted with at least one nosepiece which slides into its exactly fitting integrated guide rail in the bioreactor carcass.

45. Device according to Claims 32 - 44, characterized in that the control and steering magnet (15) situated outside the bioreactor (1) brings about an oriented movement of the implemented mini actuator (14) with the electro-) magnetic field which it generates with the north pole of the permanent magnet which is oriented upwards (22).

46. Device according to Claims 32 - 45 characterized in that the control and steering magnet (15) is medially situated in a vertical axis to the pressure stamp (14), preferably above the pressure stamp (14) and moves upwards and downwards in relation to the polarity of the mini actuator (14), resulting in an alteration of the pressure applied to the transplant (11).

47. Device according to Claim 32, characterized in that the control and steering magnet (15) comprises two permanent magnets (32) with different vertical magnetic pole directions which are inserted in a rectangular shaped magnet holder (31) and moved to their horizontal position above the bioreactor in a cyclic manner by means of a servomotor (29) and a guide rail (30).

48. Device according to Claim 32, characterized in that the control and steering magnet (15) comprises a minimum of two

permanent magnets (32) with different vertical magnetic pole directions, these being in a disk-shaped magnet holder (31) and moved over the bioreactor (1) in a cyclic manner as a result of the rotation drive of a servomotor (29).

49. Device according to Claim 32, characterized in that the bioreactors which are firmly fixed in their horizontal position approach the permanent magnets (32) via a vertical movement of the magnet holder (31) by means of a step motor, in order to increase the field effect and generate the application of higher pressures on the transplant (11).

50. Device according to Claim 49, characterized in that at least two bioreactors (11) are so arranged in a station that their mini actuators (14) are driven by just one permanent magnetic control system in a contactless manner.

51. Device according to Claim 32, characterized in that the control and steering magnet (15) is in the form of an electromagnet with at least one induction coil (35) with a field which is infinitely variable using the known means.

52. Device according to Claims 32 and 51 characterized in that die induction coil (35) is highly frequently triggered by frequencies which can be altered in order to generate high-dynamic magnetic field alterations and mini actuator (14) movement on the transplant (11).

53. Device according to Claims 13 - 53 characterized in that a seeding piston (25) which is preferably cylindrical and ahs an inside diameter which corresponds to the outside diameter of the transplants on a moving sliding plate (27) for the purpose of injecting the cells (12) and the carrier matrix (13).

54. Device according to Claims 13 - 53 characterized in

that the moving sliding plate (27) and the inside of the seeding piston (25) is coated by an inert membrane, foil or polymer fleece.

55. Device according to Claims 53 and 54 characterized in that an exactly fitting stamp (26) with a planar stamp surface or an organotypical negative form in the seeding piston (25) is lightly applied to the cell suspension.

56. Device according to Claims 53 - 55 characterized in that the outside diameter of the seeding piston (25) exactly matches the inside diameter of the bioreactor (1).

57. Device according to Claims 13 - 56 characterized in that at least 3 fixation walls (28) are integrated in the reactor floor of the bioreactor (1), these having dimensions which accommodate a transplant insert and do not impair the pressure compression.

List of Reference Drawings

1	Bioreactor
2	Medium reservoir
3	Supplement reservoir
4	Hose system
5	Circulation pump
6	3-way valve
7	4-way valve
8	Sample taking section
9	Biosensors Glu/Lac
10	Measuring heads pH, pCO_2, pO_2
11	Transplant
12	Cells/tissue
13	Support matrix
14	Mini actuator
15	Control magnet
16	Porous sinter material
17	Transparent glass
18	Permeable membrane
19	Hose coupling/Luer connection
20	Pinch ring
21	Reactor lock
22	Permanent magnet
23	Enveloping body
24	Stamp surface
25	Seeding piston
26	Stamp
27	Sliding plate
28	Fixation wall
29	Servomotor
30	Guide rail
31	Magnet holder
32	Permanent magnet
33	Flow channels
34	Extension nosepiece
35	Coil

Summary

Process and Bioreactor for the Cultivation and Stimulation of Three-dimensional, Vitally and Mechanically Resistant Cell-Transplants

The task of the invention is the creation of a process and a bioreactor for the manufacturing of three-dimensional, vital and mechanically-resistant cell cultures, by which they can be cultivated and stimulated within a short time of each other or simultaneously. The bioreactor should permit GMP-conform transplant cultivation under guaranteed sterile conditions.

The bioreactor (1) comprises a basic carcass which is connected to a reactor lock (21) so that it is pressure proof and sterile, this creating at least one reactor chamber, a storage space for a transplant (11) and a mini actuator (14) being implemented in this. The bioreactor (1) is also equipped with at least two hose coupling connections (19) for the feeding and discharging of the medium in addition to the gassing.

The invention enables GMP-conform cultivation of three-dimensional, vital and mechanically-resistant cell cultures, preferably cartilage-cell constructs which can hereby be cultivated and stimulated in a locked mini-bioreactor simultaneously, consecutively or within a time-controlled process according to GMP guidelines. These so-called transplants which are cultivated in this manner are then available as replacement tissue material for the therapy of connective and supporting tissue defects, direct joint traumas, rheumatism and degenerative joint disease, for example and can present an alternative to the conventional (operative) therapy approaches, such as micro fracturing or drill perforation in arthrosis of the knee joint, for

example.

Figure 3